A STUDY ON THE RESILIENCY OF A MUTUALISM BETWEEN CARPENTER BEES AND ANGIOSPERMS ON MOUNT LEMMON IN A CHANGING CLIMATE

By

EMILY RUTH SPINDLER

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Approved by:

Dr. Judith Bronstein
Department of Ecology and Evolutionary Biology

Abstract:

While many scientists are studying climate change's impact on abiotic factors, my interest is in studying climate change's impacts on biotic factors–specifically, to determine if ecosystem networks will be preserved or altered with abiotic change. To examine this topic, I centered my thesis around carpenter bees on Mount Lemmon and their pollination interactions with local flowers. The carpenter bees (*Xylocopa californica*) are prime candidates for study because they create pollen balls that can be easily accessed, stored, and used in pollen analyses as a proxy for pollination. Kathryn Busby, a graduate student studying the carpenter bees, preserved several provisions from 2016. This gave me the opportunity to determine the composition and diversity of the pollen, create a snapshot of the interaction between the carpenter bees and flowers in 2016, and establish a replicable procedure so that data can be collected in future years. Later studies compared with my data may determine if the families of flowers visited by the carpenter bees or the frequency with which the bees are visiting are changing over time. Monitoring over several decades could tell us if climate change has an effect on local mutualistic networks.

Background:

Introduction:

As a conservation biology major, my studies have focused on how to monitor and manage a population, and strategies to protect endangered species. I'm interested in climate change and how it might affect endangered species' populations and communities. For my honors research I decided to focus on plant-pollinator mutualisms because those interactions and pollination ecosystem services are fundamental to sustenance of life on Earth. Further study on the resiliency of plant-pollinator relationships is essential to understand how ecosystems as a whole, as well as the individual species, will be affected by climate change.

There are two main ways that climate change can impact a species, which in turn impacts the community. The first is through distributional shifts. Because of rising temperatures, a species may have to migrate polewards, or upward in elevation to stay in a livable climate or community (Chen et al 2011). The other way that climate change can impact a species is by shifting its phenology. Since temperatures are rising faster in the spring (Schwartz, Ahas, and Aasa 2006), and many plants rely on temperature and precipitation as cues for flowering (Crimmins et al 2008), climate change may impact their flowering phenology. Insects are thought to rely on temperature cues to initiate their spring emergence (Bale et al 2002). This creates the possibility of a phenological mismatch, in which the flowers bloom at a different time than the insects emerge, and the pollinator misses the optimal pollination window. This could be disastrous for both species in the mutualism because the flowers might not be pollinated, and the bees might not receive the nectar or pollen they need to survive and reproduce. This could have impacts beyond the mutualism too; for example, other animals might rely on the fruit that the flower would produce as a primary food source.

Changing phenology poses a threat to plant-pollinator relationships. A study found that in northeastern North America, phenology of ten generalist bee species advanced by 10 days over 130 years, with the majority of the advancement since 1970, lining up with increasing atmospheric CO_2 concentrations and global temperatures (Bartomeus et al 2011). In this case, the bee phenology is changing, and more information is needed about the flowering plants responding to the same cues to know if the mutualism would be severed or maintained. In another example, a model incorporated 1420 pollinator and 429 plant species and doubled the CO_2 concentration. Changing phenology made floral resources 17-50% less available to bees than they were a little over a century ago (Memmott 2007). The worst-case scenario of the model predicted half of the pollinator activity would occur when there were no floral resources whatsoever (Memmott 2007). These studies support the notion that changing phenology could lead to mismatches in mutualisms.

However, there is less local data to determine if phenology would be an issue for plant-pollinator relationships in the Sonoran Desert. David Bertelsen climbed Finger Rock trail in the Santa Catalina Mountains approximately weekly for twenty years

and took detailed notes on what plants were flowering along the trail. After analysis of Bertelsen's flowering phenology records, Crimmins et al found several complex patterns involving precipitation, temperature, and elevation that affected flowering times. At the highest elevations, diversity and composition of species in bloom are shifting later due to increasing temperatures (Crimmins et al 2008). We don't know what factors might be affecting bee phenology and if their life cycle will still match up with the plants in the Santa Catalinas.

To investigate whether rising temperatures is creating phenological mismatches, I studied the plant-pollinator relationship between carpenter bees (*Xylocopa californica*) and angiosperms at the Gordon Hirabayashi Campground on Mount Lemmon. I created a replicable procedure and took a snapshot in time of what flowers the bees pollinated in 2016 to utilize and compare to future years. With information collected over several years, we could learn if the mutualism was changing with the impacts of climate change.

Study Site:

The elevation of Tucson is about 670 meters, and Mount Lemmon is about 2791 meters, with the Gordon Hirabayashi Recreation Site at around 1524 meters (Coronado National Forest n.d.). As elevation increases, temperature decreases and precipitation increases. This creates altitudinally distinct plant communities adapted to the particular microclimate at that elevation (Mount Lemmon Highway 1994). The camp is dominated by chaparral and oak woodland plant communities. The oaks tend to grow where there is more water, and chaparral tends to grow on hillsides (Mount Lemmon Highway 1994). Other common plant species are manzanita, skunkbush, Arizona oak, Emory oak, alligator juniper, and border piñon (Mount Lemmon Highway 1994). Kathryn collected *Agave* and *Dasylirion* stalks from the grassy hill to the North of the Hirabayashi Campground.

Study Species:

Xylocopa californica has three subspecies: *arizonensis*, *californica*, and *diamesa*. *Arizonensis* has discontinuous populations in the southern Great Basin, Mojave and Colorado deserts, Arizona, New Mexico, Texas, and Mexico. *Californica* is the common green carpenter bee, and its habitat is associated with incense cedar and Sierra redwood tree distributions. *Diamesa* is the common bluish carpenter bee with wings paler than *arizonensis*, but darker than *californica*. It lives in southern and central coastal cismontane California (Hurd 1955). These subspecies nest in dead or mostly dead softwood species in those areas: *arizonensis* typically nests in *Agave*, *Dasylirion*, *Populus*, *Sequoioideae* and *Yucca*, and *californica* typically nests in *Calocedrus decurrens* and *Sequoiadendron giganteum*, and *diamesa* typically nests in *Yucca whipplei* (Hurd 1955).

Although the nesting biology of *Xylocopa californica* has not been studied, there is reason to believe that it is similar to another carpenter bee, *Xylocopa tabaniformis*.

Nininger studied *Xylocopa tabaniformis* and found that their burrows vary from four to six inches long, and that it takes six days for a female bee to lengthen her burrow one inch (Hurd 1955). Once the burrow is finished, *Xylocopa tabaniformis* uses pollen and nectar to create a pollen ball equivalent to the mass of a bee's abdomen, and then lays an egg on it. Then it creates a cell partition made of wood chips, and creates another pollen ball for another egg, and continues until the nest is full (Hurd 1955). *Tabaniformis* eggs hatch in a week, consume the pollen balls and become larvae in 22-28 days, then become adults in 40-45 days, break the partition and leave the nest (Hurd 1955). The *Xylocopa californica* life cycle is similar.

Hypotheses:

While I planned to perform pollen analyses for provisions from several years, I was not able to find preserved provisions from before 2016 from Mount Lemmon. Instead of hypothesizing how the pollen represented in the provisions might change over several years, I focused on analyzing the provisions from 2016, understanding as much as I could about the pollen the carpenter bees had collected, and creating something that could be compared to future years to learn more about how climate change might affect the mutualism between the carpenter bees and the flowering plants at the Gordon Hirabayashi Campground.

My first hypothesis states that there will be equal frequencies of all morphospecies in all pollen provisions. This information could be compared over several years to see if the frequencies of the species change. My second hypothesis stated that pollen composition of provisions are unrelated to their position in the carpenter bee nest. My last hypothesis stated there is no difference in the pollen for the two species of nest stalk that Kathryn Busby collected (Figure 1).

$H_0$1)	There will be equal frequencies for each morphospecies represented in the pollen provisions.
H_A1)	The frequencies will not be equal among morphospecies represented in the pollen provisions.
$H_0$2)	The pollen composition of provisions are unrelated to their position in the carpenter bee nest.
H_A2)	The pollen composition of provisions are unrelated to their position in the carpenter bee nest.
$H_0$3)	There is no difference between pollen composition of provisions in *Agave* nests versus *Dasylirion* nests.
H_A3)	There is a difference between pollen composition of provisions in *Agave* nests and *Dasylirion* nests.

Figure 1. Listed hypothesis.

Methods:

Field collection:

Graduate student Kathryn Busby demonstrated carpenter bee nest collection methods in the field. At Hirabyashi Campground, she checked all *Agave* and *Dasylirion* stalks around the height of the tips of the leaves for holes that indicate carpenter bee activity. When she found a stalk with a hole, she chopped down the stalk and took it back to the lab. In the lab, she split the stalk open to see if there was carpenter bee nesting activity inside. Often, there were also pollen provisions in the nest cells (See Appendix A Figure 1). She collected all of the provisions she found in the nests and preserved them in ethanol. Provisions were individually stored in vials with records of which cell they occupied, while others were stored together in one vial for a whole nest of provisions. Notes on the vials included in which species of stalk the nest was found, with a letter A for Agave and D for Dasylirion, and the date that she found the nest.

Lab procedure: Creating the slides

I adapted my lab procedure from methods described in Jones 2012. I analyzed six vials of pollen provisions weekly. I first labeled the vial caps with one to six dots that matched my labeled paint pallet wells (See Appendix A, Figure 2). I opened the first vial and used forceps and a dissecting needle to extract a small chunk of the provision and put it in the corresponding paint pallet well labeled with one dot. Then I used an eyedropper to extract some of the ethanol-pollen solution in the vial and add it to the chunk in the paint pallet well. I used the dissection needle to mix the chunk into the solution to make a relatively homogenous mixture. I cleaned my tools and repeated the process for the rest of the vials. I used a vortex to more thoroughly homogenize each sample. I then allowed the mixtures to dry in the paint pallet. Sometimes, the provision was dry due to ethanol evaporation. In this case, I still tried to break off a small chunk of the provision and soaked it with some ethanol in the well. I wet it first with the alcohol to try to avoid volatizing pollen grains. I then attempted to crush the provision chunk into smaller bits manually and with the vortex and wait for the ethanol to dry. In either case, as the ethanol evaporated, I swirled the mixture around the pallet well to help it dry faster.

While the ethanol dried, I labeled my microscope slides with the information written on the vial, as well as the number of dots, the date processed in the lab, and my initials. When the ethanol evaporated to leave a sticky film on the pallet, or the mixture was at least reduced to a glue-like consistency, I used the dissecting needle to pick up Fuchsin jelly cubes (prepared by the Bronstein lab) and collect the pollen substance. I swiped with my jelly cube from the center of the pallet well to the rim to try to account for gravity. (Bigger pollen grains might have collected in the bottom of the well, whereas smaller pollen grains might have stuck to the edges of the well.) I placed the jelly cube with the pollen about a centimeter from the top of the corresponding slide. I repeated this process for each slide. I then lit an alcohol lamp

and held the first slide over the flame briefly to melt the jelly cube. I found that it was most helpful to remove the slide from the flame when there was still a little jelly left in the cube form because the remaining heat would melt the rest but prevent the jelly from bubbling too much. If the jelly bubbled, I gave it a few seconds to cool down because when the cover slide was applied while the jelly was too hot, it bubbled up and the cover slide moved or fell off completely. Once I applied the cover slide to the jelly I turned the slide over and laid it down on a Kimwipe to cool and solidify. I repeated this process with the rest of the samples (See Appendix A Figure 3).

Lab procedure: Photographing pollen slides

Once all the slides were cool, I loaded the Leica Application on the computer and turned on the microscope and the Leica control panel. I placed the first slide right side up on the microscope stage and used the program on the computer to view it. I used the control panel to zoom in until I could see the 200 micron bar and put the pollen into focus. My typical slide landscape had a high concentration of pollen in the middle, declining in abundance from the center (See Appendix A Figure 4). Also, larger pollen grains might have had a tendency to be in the more concentrated area of the slide than near the edges because they would have had more mass and taken more energy for the jelly to move when it melted than smaller pollen grains. Generally, I took pictures of the slide in places where the pollen was less concentrated so that I could adequately count and identify the pollen grains. This might have skewed my results to have fewer large pollen grains. I took three pictures of distinctly different-looking communities of the slide if possible to best represent the pollen on the slide. I repeated this process for every slide, then exported the pictures to my flash drive to count and identify them on my own computer.

Lab procedure: Counting and Identifying Pollen Grains

Pollen is notoriously difficult to identify (Jones 2012), so for my project, I assigned my pollen to morphospecies. I started with a few different morphospecies, and ended with eight distinct morphospecies (See Appendix B for descriptions). This was because I didn't see all of the different morphospecies in the first few slides. Some of the morphospecies were very rare and only showed up in a few photos. However, I also suspect that I improved at distinguishing pollen grains and I might have identified separate morphospecies later, after I had already counted and classified some of them as the same. It would have been beneficial if I had re-classified all the pollen in my photos after I had set all the categories, to improve my accuracy. To count and classify the pollen, I started in one corner of the photo and used a tally counter to count the number of "Small" pollen grains I saw (this category was consistently the most abundant). Then I counted all the other morphospecies. On an Excel spreadsheet, I recorded the information on the slide, the number of pollen in each morphospecies that I saw in each of the three photos

per slide, and any additional notes. I coded my spreadsheet so that it would automatically compute the proportions when I entered all the numbers.

Statistical analysis:

Once I had collected all my data, I needed to organize it and analyze it to see if it rejected my several hypotheses. My first hypothesis stated that the frequencies of all pollen types were equal. I calculated the frequency of each morphospecies for each pollen provision. Then I added the frequencies for all the provisions and divided by the total number of provisions to get the mean frequency for each morphospecies. I performed a chi-squared test to determine if my first hypothesis would be rejected. I then made a pie chart to display the mean frequencies of each morphospecies. The other two hypotheses required that I perform a Non-metric Multi-Dimensional Sampling (NMDS) analysis in R to quantify how similar provisions were to one another within and between nests. I reorganized my data into four excel spreadsheets: three spreadsheets with the data from the nests A1, D2, and D3, and one with data from all nests (I averaged the frequencies from the A1, D2, and D3 nests to use for this dataset). I uploaded these to R and performed the analysis. I reduced the matrix to two dimensions and used a trymax of 100 to reduce stress. I created NMDS plots using the ordiplot function and labeled my species and provisions or nests depending on the test. Using this method, I was able to perform the analysis on nest A1 and ALLNESTS, but I was not able to perform the analysis on nests D2 and D3 for a lack of data.

Results:

A pollen analysis was performed on 66 pollen provisions, and 28,660 pollen grains were counted and identified in this study. My analysis examined the frequencies of the morphospecies present in all of the pollen provisions I analyzed. The "Small" morphospecies had the highest frequency, the "Peppermint Candy" morphospecies had the next highest frequency, but was much lower than the "Small" category. The third most common morphospecies was called "Triangle". The rest of the categories were rounded to less than one percent. My first hypothesis predicted there would be equal frequencies for each morphospecies represented in the pollen provisions. The Chi-squared test returned a p-value of 2.9x10^-18 indicating that my hypothesis should be rejected at any reasonable significance level (Figure 7).

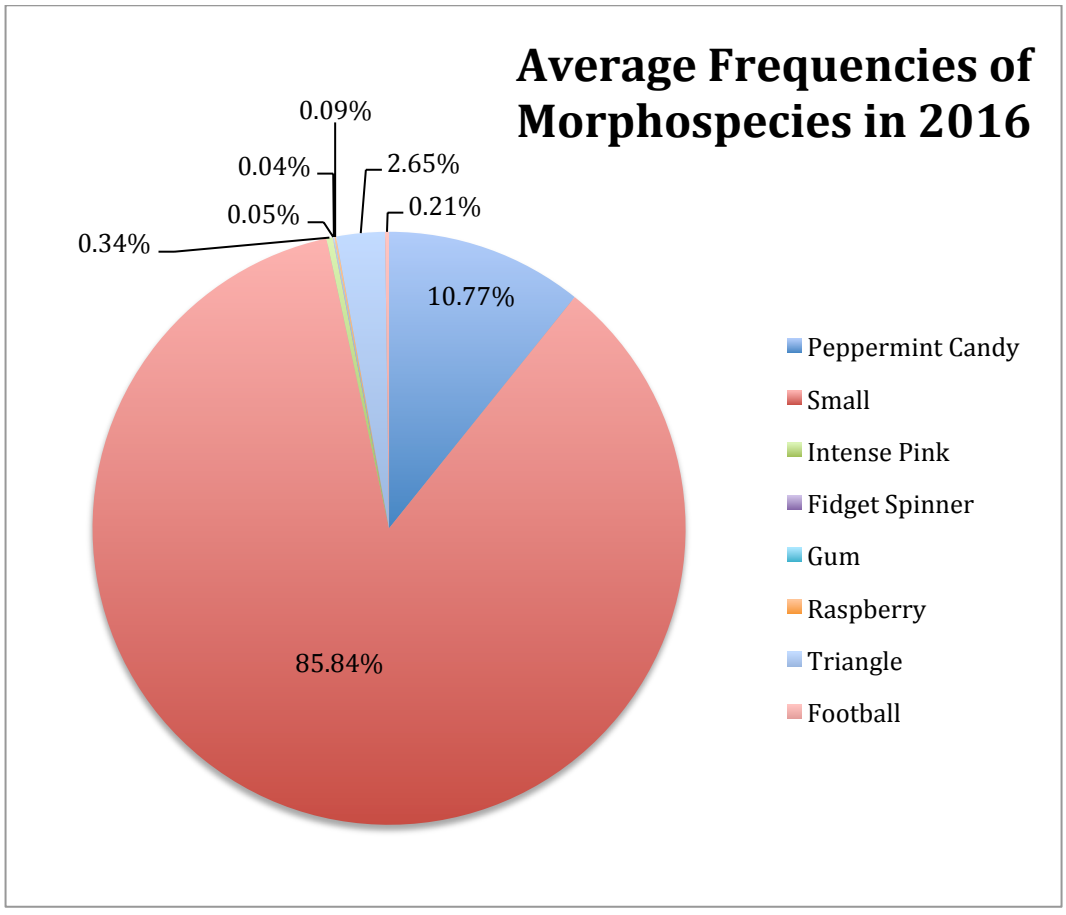

Figure 4. Pie chart showing the averaged percentages of morphospecies from all provisions with morphospecies listed in a legend.

Non-Metric Multidimensional Scaling ranks dissimilarity among the samples and creates a plot that visualizes dissimilarity as distance. For the nest A1 collected on May 11th 2016, I analyzed twenty pollen provisions. The NMDS plot (Figure 5) reveals that most of the provisions are clumped together and are not dissimilar. However, the provisions in the nest locations UP CELL 5, UP CELL 6, and UP CELL 7 are further away from the rest of the provisions. These had more pollen grains from the "Raspberry" and "Triangle" morphospecies. My second hypothesis that the provisions could not be connected with their position was not rejected (Figure 7).

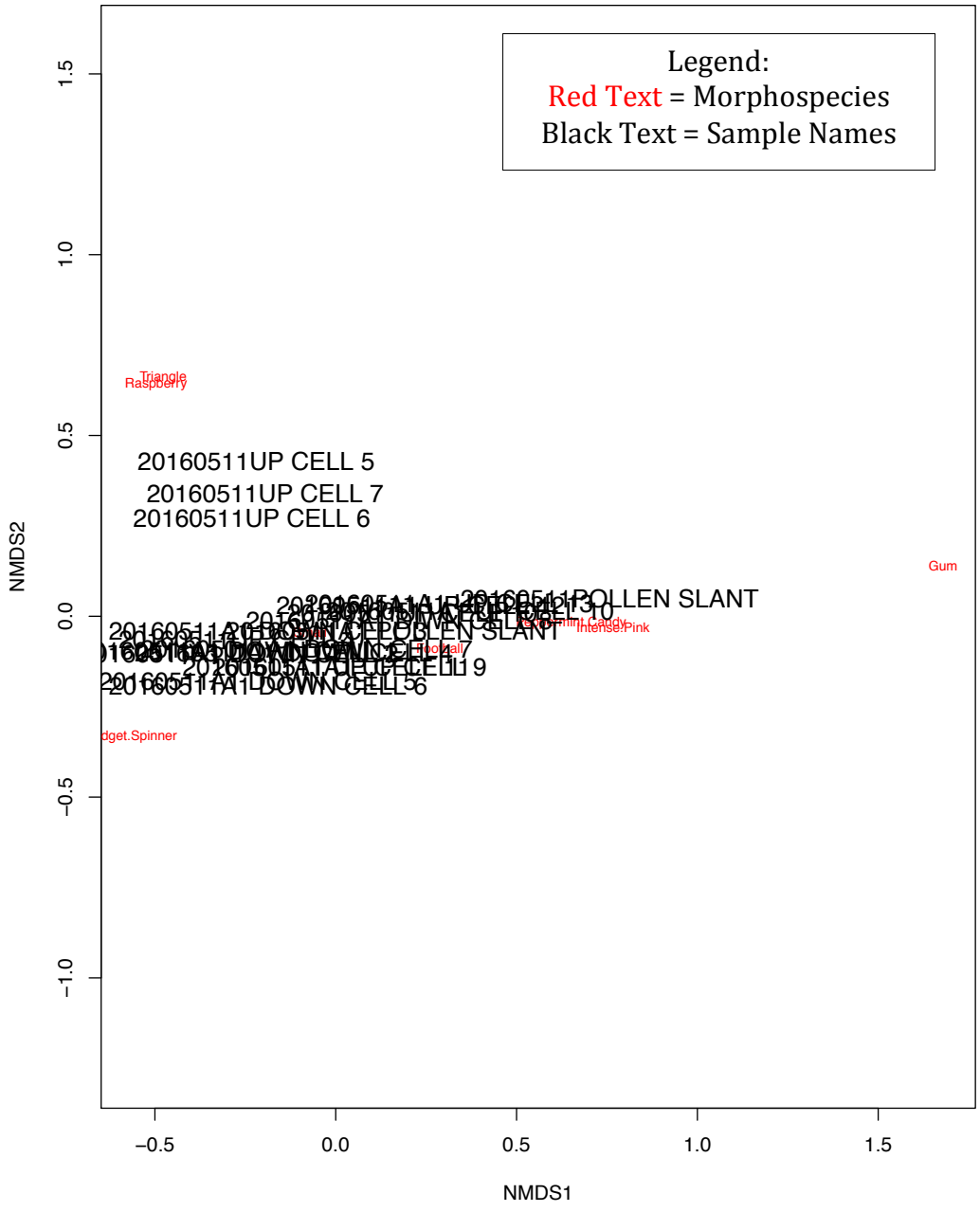

Figure 5. NMDS analysis shows that most of the provisions are similar.

In the NMDS plot for ALLNESTS (Figure 6), most of the nests are clumped together and not dissimilar. The nest 20160608D1 is furthest away from the cluster of similar nests. It is also closest to the "Gum" morphospecies. My raw data show that this is one of the only provisions that had the "Gum" morphospecies in it. However, the nest 20160810D3 did not have any "Gum" pollen grains. The nests 20160518D6 and 20160518D8 were further away from the cluster of nests and closer to the "Peppermint Candy" label. This indicates that they had a higher frequency of the "Peppermint Candy" morphospecies than the other nests. The NMDS plot for ALLNESTS did not show a difference between the morphospecies represented in *Agave* and *Dasylirion* nest stalks (Figure 7).

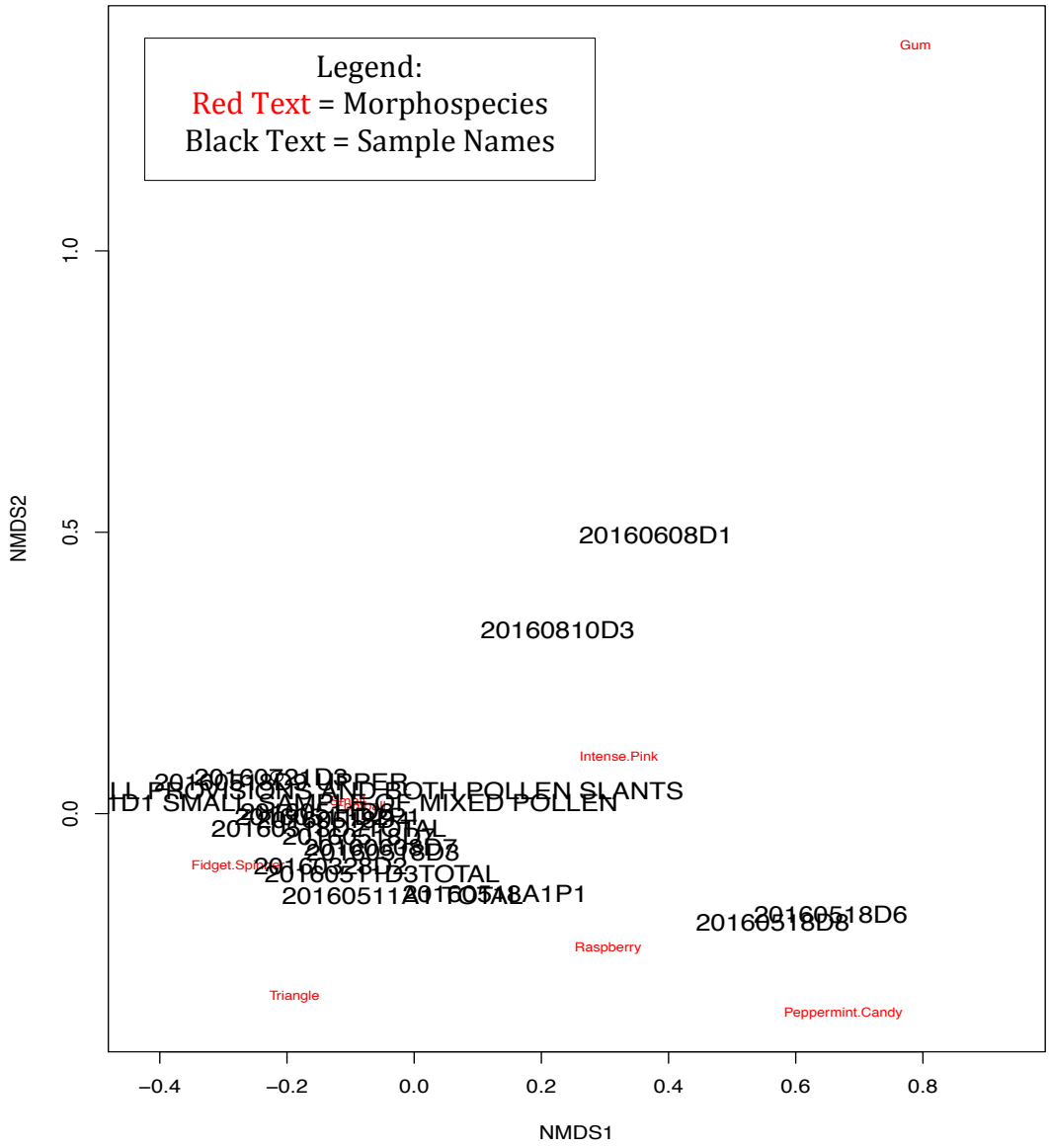

Figure 6. NMDS analysis shows that most of the nests' provisions were similar.

The pollen provisions collected in 2016 revealed eight morphospecies with frequencies dominated by the "Small" category. The nest A1 dataset showed that most of the provisions were similar, with a few outliers that were closer to the "Gum" morphospecies. The ALLNESTS dataset showed that most of the nests were similar, with a few outliers that included more of the morphospecies "Peppermint Candy", "Raspberry", and "Triangle" than the others.

$H_0$1)	There will be equal frequencies for each morphospecies represented in the pollen provisions.	Rejected
H_A1)	The frequencies will not be consistent among morphospecies represented in the pollen provisions.	Not Rejected
$H_0$2)	The provisions cannot be correlated with their position in the carpenter bee nest.	Not Rejected
H_A2)	The provisions are correlated with their position in the carpenter bee nest.	Rejected
$H_0$3)	There is not a difference between the pollen in *Agave* nests versus *Dasylirion* stalks.	Not Rejected
H_A3)	There is a difference between the pollen in *Agave* nests versus *Dasylirion* nests.	Rejected

Figure 7. Hypotheses chart with results.

Discussion:

My data could be used to determine whether the mutualism between carpenter bees and flowering plants on Mount Lemmon is changing over time with climate change by adding more years of monitoring morphospecies frequencies. Studying bees and flowering plants is important because pollination is an essential ecosystem service for ecosystem function and human survival. We need to know if these mutualisms are resilient or if they will dissolve due to phenological mismatch, and if there is anything we can do to mitigate for the change.

Also, the data collected using my procedure can tell us more about the carpenter bees and flowers. In the NMDS Plot of ALLNESTS, (Figure 6) two nests were further away from the cluster of nests and closer to the "Peppermint Candy" label. This suggests that perhaps bee behavior and choice of flowers to visit influences the pollen in their nests. In the NMDS Plot of nest A1 (Figure 5), the provisions in the nest locations UP CELL 5, UP CELL 6, and UP CELL 7 were further away from the rest of the provisions and closer to the "Raspberry" and "Triangle" morphospecies. Some bee experts generalize that it takes bees one day to make one provision, and this might suggest that the "Triangle" and "Raspberry" morphospecies might have had a concentrated flowering event in a few days, when the bee created those provisions. The data collected using my procedure could answer questions about flowering phenology and bee behavior as well as contribute to climate change research.

Future Improvements:

I could have done several things to make my procedure more robust. If I could do this project again, I would have tried to use a pollen reference collection to determine the pollen families instead of analyzing all of the pollen into morphospecies. I used the category "Small" for pollen grains that were relatively smaller than the rest, but higher resolution photographs showed that the small category actually included several distinct kinds. Some of them had patterns similar to the "Peppermint Candy" pollen grains, and there was more variation in size. If my "Small" category had been split into several morphospecies, there might have been more diversity captured in my study. Also, my counts might be inaccurate for samples processed at the beginning. For example, the "Triangle" category was invented later than the "Peppermint Candy" category, and triangle-shaped pollen grains may have been classified as "Peppermint Candy" because I didn't think it was distinct or prevalent enough to make a new category. This could have led to inflated "Peppermint Candy" proportions and lower "Triangle" proportions. There also could have been some procedural bias. While framing the photos of the slides under the microscope, I took pictures where there was lower concentration of pollen grains to accurately identify and count them, but because gravity might have clustered all the heavier pollen grains towards the concentrated center of the slide, I might have counted more of the smaller morphospecies than the larger ones. While these errors might not have changed whether my hypotheses were rejected, it is important to have accurate proportions so that they can be compared to future years to see if

climate change is impacting the diversity and abundance of pollen collected by the carpenter bees.

If this experiment were continued, I would consider spending the time to identify the pollen to family level. If not, I would add more morphospecies or divide my existing morphospecies into more categories, as I have learned how to better distinguish between the pollen grains. I would also suggest counting and identifying the pollen while at the microscope instead of taking photographs and identifying the pollen later. This would allow zooming with more resolution. If I did continue to take photographs of the pollen, I would also implement some randomized or mechanized method to take the pictures to eliminate any procedural bias.

Conclusions:

Comparing several years of this data would determine if the frequencies of the morphospecies are changing over time with increasing effects of climate change. If some morphospecies were no longer represented in the pollen provisions after several years of data collection, this might indicate that the bees are no longer pollinating that kind of flower, which could be the result of a phenological mismatch. Also, if there was decreasing diversity of morphospecies in the pollen provisions over several years, this could be a clue that fewer species of flowers are being pollinated at the Hirabayashi Campground, which could be further supplemented with plant phenology records. Developing this record could add to our knowledge of what flowers are blooming when on Mount Lemmon, show us bee behavior patterns in pollination, and help us learn more about *Xylocopa californica* and their nests in a changing world.

Acknowledgments:

I couldn't have done my thesis without Kathryn Busby, who not only collected all of the provisions from 2016 in the field, helped me develop my procedure, and helped me with the statistical analysis, but supported me through the entire process. Judith Bronstein helped me develop my thesis topic and procedure in the early stages and guided me through the process. I also learned so much from her lab and I am grateful for my time there. A special thank you to Goggy Davidowitz and Heather Costa for letting me borrow their microscope and sharing their lab with me. I'm so fortunate to have the resources available to me and the people to encourage me to pursue climate change ecology research!

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Appendix A: Photographs

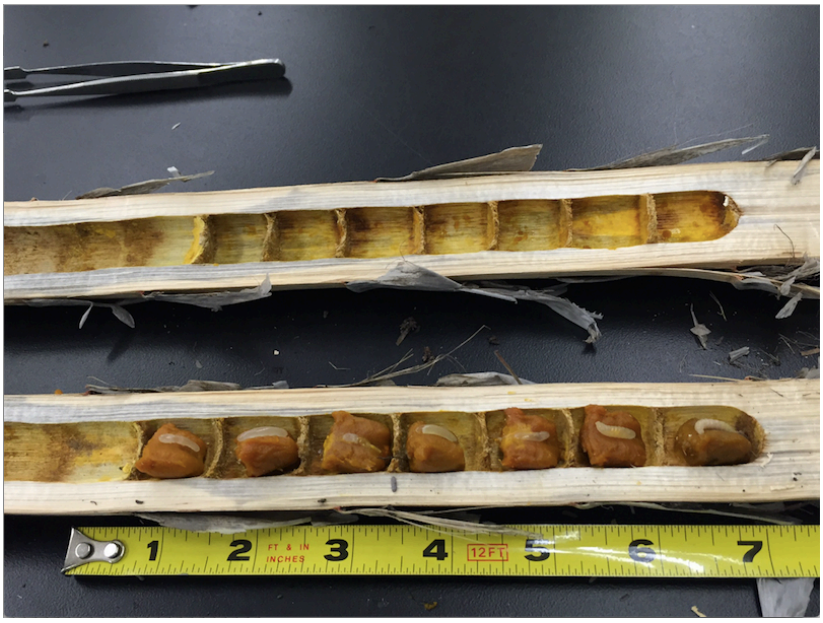


Figure 1. Pollen provisions in nests with larvae and eggs on them.

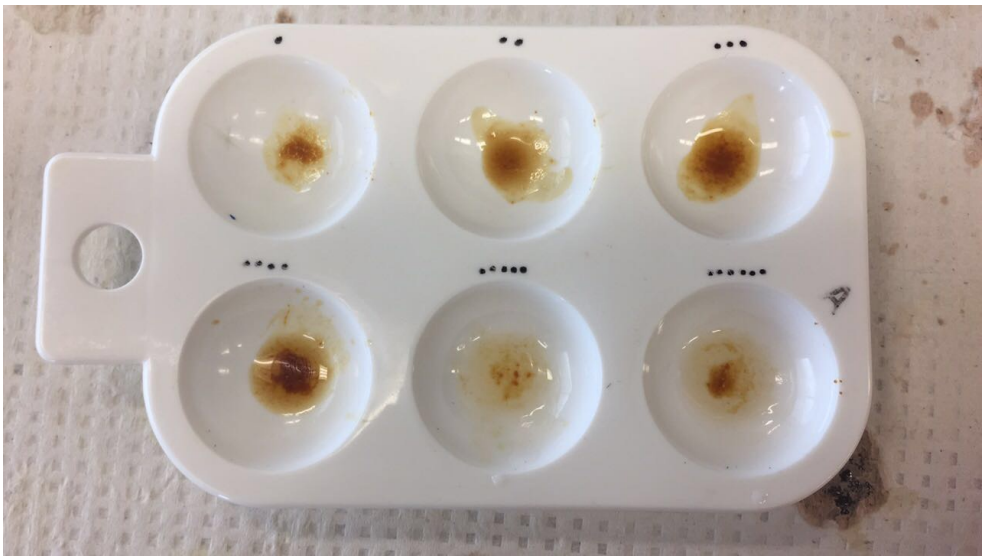


Figure 2. Paint pallet with homogenized pollen provision chunks.

Figure 3. Slides with pollen suspended in Fuchsin jelly.

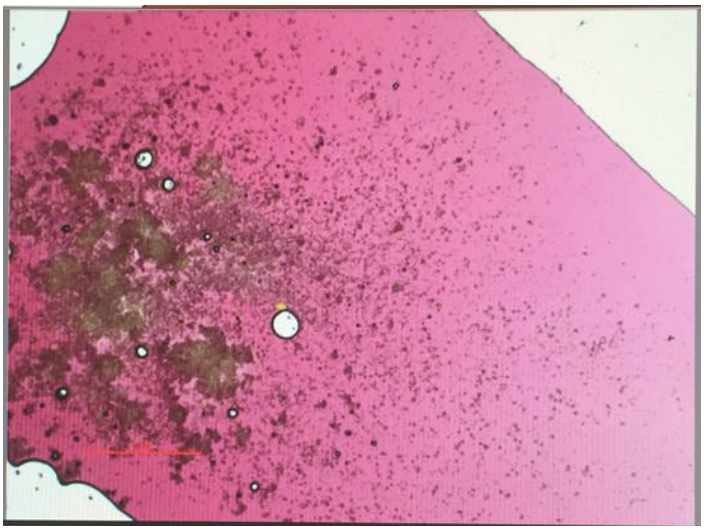
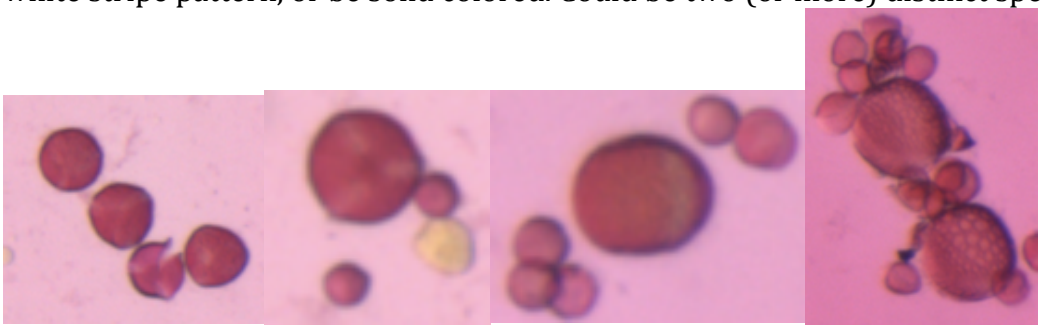


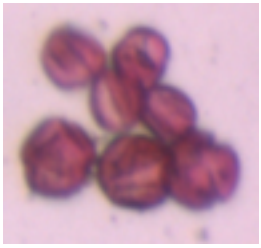
Figure 4. Zoomed out microscope view of a slide. Areas with high pollen concentration appear lighter brown.

Appendix B: Pollen Morphospecies Descriptions

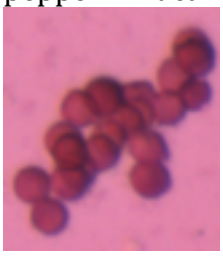
Peppermint Candy: Round shape, relatively large, can have white pinwheel pattern, white stripe pattern, or be solid colored. Could be two (or more) distinct species.



Extra Small: Smallest morphospecies, textured, look like raisins, can occur in clusters. I deleted this category because it was too difficult to distinguish from "Small". I should have combined the counts that I had for both categories.

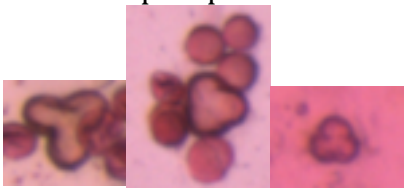


Small: Generic small morphospeices, most common, can occur in clusters. Most likely several different species, with closer inspection they have patterns like the peppermint candy pollen and a variety of sizes.

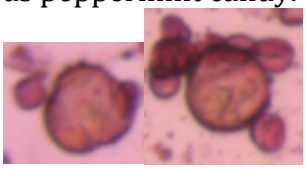


Intense Pink: More intense pink color, slightly larger than the small morphospecies, darker outline, more transparent.

Fidget Spinner: Distinct three petals, shaped like fidget spinner. Usually size of extra small morphospeices.



Gum: Off-colored, yellow-ish, dirty texture, looks like gum that's been stuck on the sidewalk for a while, usually not a perfect oval like the peppermint candy, same size as peppermint candy.



Raspberry: Spiky all over, some have pronounced three knobs. Could be two separate species.

Triangle: Some more conspicuous than others but all have the triangular shape with three knobs, slightly larger than the small morphospecies. This could be two different species.

Football: Intense pink coloration, but with two indentations that make it look like a deflated football, slightly larger than small.

Unknown: I only saw this pollen grain once or twice so I didn't make a new category for it but it was much larger than all of the pollen grains I've seen.